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Review

Why modules matter ☆

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ABSTRACT

The serendipitous discovery of the SH2 domain unleashed a sea-change in our conceptual molecular understanding of protein function. The reductionist approaches that followed from the recognition of modular protein interaction domains transformed our understanding of cellular signal transduction systems, how they evolve and how they may be manipulated. We now recognize thousands of conserved protein modules – many of which have been described in structure and function, implicated in disease, or underlie targeted therapeutics. The reductionist study of isolated protein modules has enabled the reconstruction of the protein interaction networks that underlie cellular signalling. Protein modules themselves are becoming tools to probe cellular activation states and identify key interactions hubs in both normal and diseased cells and the concept of protein modularity is central to the field of synthetic biology. This brief word of introduction serves to highlight the historical impact of the very powerful idea of protein modules and sets the stage for the exciting on-going discoveries discussed in this issue.

1. The SH2 domain that started it all

The early 1980's witnessed an explosion in our understanding of cellular signaling that flowed from the discovery of tyrosine phosphorylation [1] and the implication of tyrosine kinases in transformation, growth and proliferation [2–5]. Yet the means by which tyrosine kinase exert their effects and how different receptors maintain specificity remained ambiguous. Transphosphorylation of kinases as a means of activation was well established but the search for downstream targets produced the surprising result that the most abundantly Tyr-phosphorylated protein in growth factor stimulated cells is commonly the receptor itself. To Tony Pawson and co-workers this "suggested that tyrosine phosphorylation might have unsuspected biochemical properties, and that the kinases might have ways of recruiting their targets in addition to the transient binding of a substrate to the active site of the enzyme" [6]. The pursuit of this idea lead to the identification of the Src homology 2 (SH2) domain as an independently folding domain of certain non-receptor tyrosine kinases that regulated kinase activity [7–9]. In 1988, Tony Pawson wrote suggesting that signaling by modular interactions might be a more general principle [10]. This principle was further expanded by the pioneering work of Hidesaburo Hanafusa and Bruce Mayer who cloned the Crk adaptor protein composed exclusively of an SH2 domain and a second non-catalytic domain termed the SH3 domain. Isolated SH2 domains were found to bind to tyrosine-phosphorylated proteins and directly able to interact with phosphotyrosine (pTyr) sites [11–15]). The first structural description of an SH2 domain revealed how it recognizes the pTyr moiety of a phosphopeptide and brought to light several features that are now recognized as common themes of modular interaction domains [16].

The SH2 domain has its N- and C-termini juxtaposed in space and removed from the ligand-binding face so that it may plug into an existing polypeptide without necessarily perturbing the surrounding structure. This feature of many modular domains suggests an evolutionary mechanism for their rapid expansion and deployment into novel proteins may help explain the development of the varied array of SH2 domain proteins in the Unikont branch of the Eukaryotes [17]. While domains have expanded in various ways in different lineages, the undeniable power of independently folding functional subdomains can be seen in the observation that most proteins encoded in the human genome contain one or more identifiable domains [18].

2. Selectivity of interactions

The extent of SH2 domain binding specificity became evident with early proteomic approaches using degenerate peptide

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libraries [19]. Most SH2 domains recognize residues C-terminal to the pTyr in a manner that varies from one domain to another and that allows general classification of SH2 domain binding motifs. Binding motifs identified *in vitro* using peptide libraries reliably mirror SH2 domain binding sites *in vivo* [20]. This opened the door to an extensive array of reductionist biochemical and proteomic studies to describe domain function and to the bioinformatic prediction of modular protein–protein interactions [21]. In this issue, Reimand et al. discuss the state of the art in predicting interaction networks formed around peptide recognition modules. Structural studies identified a highly conserved binding pocket for the pTyr residue in which the phosphate is coordinated by an invariant arginine residue in the SH2 domain. A second, more variable binding surface, engages residues C-terminal to the pTyr and thereby accommodates selectivity for specific peptide motifs [22,23]. The concept of primary and secondary binding contacts has proven paradigmatic of other families of protein interaction domains (PIDs) that bind to a conserved peptide element while achieving specificity through the variable recognition of flanking residues. Thus most SH2 domains recognize pTyr, many SH3 domains recognize PXXP, and a number of FHA domains recognize phosphothreonine etc., while individual domain selectively is dictated by the recognition of surrounding residues [24–26]. Later in this issue, Joan Teyra and co-workers discuss the use of phage display to map selectivity of PDZ and SH3 domains while David Gfeller discusses the specificity landscapes of peptide recognition modules. In peptide library screens of SH2 domains it was apparent that both permissive and disfavoured or non-permissive residues contributed to binding motifs [19,20]. This feature turns out to be critical to developing selective interactions as many SH2 domains share a preference for general motifs and anti-motif information can define distinct subsets of ligands bound by one and not another [27]. The concept of the language of peptides read by SH2 domains is the subject of the review by Liu and Nash in this issue, while Ylva Ivarsson discusses the plasticity of PDZ domain interactions and how this versatile domain can be regulated in various ways. Kalle Saksela and Perttu Permi present a review of SH3 domain specificity with a focus on non-consensus binding events while Simin Rahighi and Ivan Dikic discuss selectivity of ubiquitin-binding modules.

3. Reconstructing networks at a systems level

The ability to analyse domains *in vitro* to understand their structure and function and then to reliably apply this knowledge back to infer function in intact proteins and their role in signalling networks has been a defining feature of the incredibly powerful reductionist approach. Over and over again the insights gained from studying isolated components have informed and expanded our understanding of otherwise inaccessibly complex cellular systems. The relevancy of the reductionist approach to study PIDs has been proven time and again in their ability to explain *in vivo* behavior of proteins, signal transduction networks and even physiological behavior of cells and systems. Given the complex issues associated with more "physiological" experiments, it is not surprising that much of our mechanistic understanding of protein–protein interactions is based upon reductionist approaches and that both the design and interpretation of cell-based experiments commonly relies upon prior *in vitro* studies of the component parts. From detailed studies of individual proteins to large-scale analysis of interaction specificity, reductionist approaches based on the concept of modular protein interaction domains form the basis of much of modern biochemistry, molecular and cellular biology. Volkmer et al. detail the use of synthetic peptide arrays for the investigation of protein interaction domains in this issue while Elisabeth Le Rumeur and co-workers summarize the many uses of spectrin repeat domains. Modular interaction domains are now proving invaluable tools for probing cellular activation states. Jadwin et al. extend this concept to define "domainomics" and the variety of proteomic approaches that draw upon the concept of modular interaction domains and their defined ligand motifs. Domains are also useful reagents in proteomic studies as well as in the interpretation of proteomic data. Anne-Claude Gingras and Brian Raught present an update on the use of quantitative mass spectrometry to reconstruct protein–protein interactions and networks.

4. Domains in human disease

SH2 domains and tyrosine kinases co-evolved [17] and display remarkable synergy. This can inform therapeutic development and our understanding of drug action. The kinase inhibitor Imatinib (Gleevec) selectively recognizes a conformation of the autoinhibited Abl catalytic domain that is imposed by the adjacent SH2 domain [28]. An engineered Abl SH2-binding fibronectin type III monobody that disrupts the SH2 domain-kinase interface inhibits Bcr–Abl kinase activity and induces apoptosis in CML cells [29]. Disruption of the SH2-kinase interface also increases sensitivity of imatinib-resistant Bcr-Abl mutants to a range of tyrosine kinase inhibitors [29].

While a number of SH2 domain inhibitors have been developed, none are thus far clinically successful. Yet the concept of developing inhibitors of protein–protein interactions that target modular interaction domains is now gaining traction. Members of the Bcl-2 family are composed of Bcl-2 homology (BH) domains, which come in four flavours (BH1–4). The Bcl-2 family of proteins bind to pro-apoptotic terminal effector proteins Bax and Bak and in doing so block the intrinsic apoptotic pathway. Activated stress pathways disrupt these restraints by BH3-only proteins (e.g. Bad, Bim, etc.), which competitively displace Bcl-2-like proteins and thus release Bax or Bak to form homo-oligomeric pores in the mitochondrial membranes and initiate apoptosis. Inhibitors developed to mimic BH3 domain interactions with Bcl-2 family disrupt the protective effects of Bcl-2 family and induce apoptosis of cells via the intrinsic pathway [30]. So-called BH3-mimetics are small molecules developed to utilize multiple binding peptide-binding contacts that are broadly analogous to the multiple contact regions on many protein interaction domains. Several putative BH3-mimetics have been extensively characterised and are undergoing clinical evaluation [31]. These compounds clearly show the potential for developing effective and specific inhibitors of protein–protein interaction domains. The trick to developing such compounds has been a move away from simple screening of small molecule libraries of limited diversity and instead relies on a semi-rational approach of using nuclear magnetic resonance (NMR)-based screening, parallel synthesis and structure-based design [32]. In principle, this approach may be effective for many protein interaction domains that utilize multiple low-affinity contacts and may be fine-tuned to be highly selective using mimics of non-permissive residues to block off-target effects.

Eric Haura discusses SH2 domains as markers for normal or perturbed signalling networks and the potential use of such data to predict therapeutic response and tailor therapies for personalized medicine. Marius Sudol and co-workers discuss the WW domain of the Golabi-Ito-Hall Syndrome Protein PQBP1 and how a point mutation in the WW domain results in loss of binding function that underlies some cases of X chromosome-linked intellectual disability disorder. Panagis Filippakopoulos and Stefan Knapp discuss the Bromo-domain that recognizes acetyl-lysine and present new targets for the development of specific protein interaction inhibitors.

5. Synthetic biology and the future

The emerging field of synthetic biology builds extensively on the foundation that proteins are modular and that protein interaction domains can be swapped into novel polypeptides while maintaining their native conformation and activity. Early work on the modular interaction domains such as SH2 and SH3 laid the groundwork for much of modern synthetic biology. Pawson and co-workers showed that single residue alterations in the specificity pocket of an SH2 domain can not only change the binding properties of the domain *in vitro* but also it's biological activity in an intact organism [33] and that the fusion of the SH2 domain of adaptor proteins to a death effector domain of the FADD adaptor rerouted mitogenic growth factor signalling to induce caspase activation and cell death [34]. Such chimeric adaptors could be used to selectively kill oncogenic cells in which RTK activity is deregulated, suggesting the potential for rewiring cancer cells using synthetic biology approaches as a means of therapy. The concept of interrogating biology by reconstructing constituent components and rearranging networks is proving a powerful tool to unravel the organizing principals or networks and signalling systems (reviewed by [35]). Indeed, our understanding of networks and network evolution continues to come from studying defined protein interaction domains such as SH3 domains [36]. In this issue Marc Lewitzkey et al. discuss the molecular architecture of complexes and networks. Klaus Scheffzek and Stefan Welti summarize the PH domain while Catherine Qiurong Pan and Boon Chuan Low discuss the BCH domain. Raymond Birge delves into the C-terminal SH3 domain of Crk and it's binding and regulation. Going forward, the reductionist approaches that have followed from a modular understanding of protein architecture continue to illuminate our mechanistic understanding of cellular processes. Sachdev Sidhu makes a compelling case for a next iteration affinity reagent technology and a goal of developing genome-wide affinity reagents. Bruce Mayer provides a glimpse of dynamic protein interactions as signal transduction unfold in live cells and Gianni Cesareni and co-workers describe the human phosphatase interactome. Studies of increasing scale, systems level analysis and deep understanding of the mechanisms by which dynamic protein interactions guide cellular behaviour continue to challenge and enlighten our understanding of modular protein interaction domains.

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